Chemical interference along the Mediterranean dune gradient: extracts, BVOCs, and litter legacy effects

Leonardo Llorens

lleonard.llorens@uib.es

University of the Balearic Islands

Bartolomé Pizá

University of the Balearic Islands

Lorenzo Gil

University of the Balearic Islands

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Abstract

Chemical ecology plays a pivotal role in shaping Mediterranean dune plant communities, yet its contribution remains less explored than that of abiotic factors. This study evaluated the allelopathic potential of *Teucrium dunense*, *Helichrysum stoechas*, and *Crucianella maritima*—three characteristic species of Western Mediterranean backdunes—through germination bioassays using aqueous extracts, exposure trials to BVOCs (biogenic volatile organic compounds), and chromatographic analyses of the volatile fraction of leaf litter over a 300-day period. *T. dunense* extracts exhibited the strongest inhibitory activity, reducing the germination of *H. stoechas* and *C. maritima* by over 60% at the highest concentrations, while demonstrating high self-tolerance. In contrast, *H. stoechas* displayed significant autotoxicity, whereas *C. maritima* emerged as the most susceptible species. BVOCs emitted by *T. dunense* also suppressed recipient species' germination. Chemical analysis revealed a profound temporal transformation of the litter, notwithstanding a remarkable persistence of phytotoxic activity. Collectively, these results indicate that chemical ecology significantly drives zonation and coexistence patterns in Mediterranean dunes, with direct implications for the conservation and management of EU habitats 2210 and 2260.

1. INTRODUCTION

Mediterranean dune ecosystems are shaped by pronounced environmental stress gradients that dictate the distribution and functional assembly of plant communities (Del Vecchio, 2015). This spatial heterogeneity, driven by both natural and anthropogenic forcing, is a hallmark of European coastal systems (Acosta et al., 2009; Feola et al., 2011; Ciccarelli, 2014). While zonation is traditionally attributed to abiotic constraints—such as substrate instability, salinity, and water deficit—emerging evidence suggests that biotic filters, particularly chemical ecology, are fundamental to community structuring (Prisco et al., 2016; Malavasi et al., 2016). In resource-limited arid and semi-arid environments, secondary metabolites act as critical mediators of germination, establishment, and species coexistence (Cipollini et al., 2012; Khan et al., 2025).

Allelopathy represents a primary mechanism of chemical interference. The release of bioactive compounds—via leaching, litter decomposition, or biogenic volatile organic compound (BVOC) emissions—can significantly influence the physiology of neighboring taxa and shift community trajectories (Cheng & Cheng, 2016; Scavo & Mauromicale, 2021). Notably, BVOCs have gained prominence as dynamic mediators of plant–plant interactions under environmental stress (Jin et al., 2023; Kigathi et al., 2019). Their specific volatility and reactivity profiles contribute to the fine-scale spatial organization of vegetation. The biochemical fingerprint of dominant Mediterranean aromatic shrubs, such as *Helichrysum stoechas* L. and *Teucrium* spp., is characterized by diverse mono- and sesquiterpene profiles with known phytotoxic potential (Tsoukatou et al., 1999; Ascensão et al., 2001; Kurtoğlu & Tin, 2017).

These dunes exhibit a distinct successional zonation, harboring diagnostic communities of EU Habitats 2210 and 2260 (Martínez & Psuty, 2004; Gómez-Serrano & Sanjaume, 2009; Gracia et al., 2009). In the more stabilized backdune, where interspecific competition intensifies, chemical interference may elucidate the patterns of dominance and exclusion observed in complex plant mosaics (Ciccarelli, 2015). However, integrated studies that simultaneously examine aqueous extracts, volatile emissions, and long-term leaf litter dynamics remain sparse in Mediterranean systems.

The temporal dimension of these interactions is crucial. Litter decomposition entails substantial chemical shifts that can either attenuate or amplify the biological activity of secondary metabolites (Zhang et al., 2008; Mishra & Kumar, 2016). The biochemical quality of plant debris, rather than simple stoichiometry, often governs its ecological legacy (Hättenschwiler & Jørgensen, 2010; García-Palacios et al., 2013), particularly in terpene-rich species where degradation yields persistent oxygenated derivatives (Kainulainen & Holopainen, 2002). Understanding this "litter legacy" is essential for interpreting successional trajectories and habitat resilience.

This study investigates three representative backdune species—*T. dunense* Sennen, *H. stoechas*, and *C. maritima*—reflecting divergent ecological strategies along the stabilization gradient. We aim to evaluate their allelopathic potential through a three-pronged integrated approach: (i) germination bioassays with aqueous extracts, (ii) exposure trials to live-plant BVOC emissions, and (iii) a high-resolution temporal analysis of the litter's volatile fraction. This framework seeks to link specific chemical profiles with mechanistic interference patterns, providing a robust basis for the conservation and restoration of Mediterranean dune habitats under increasing anthropogenic pressure (Pinna et al., 2022).

2. MATERIALS AND METHODS

2.1. Plant material, study area, and sampling

Three representative species from Mediterranean backdunes were selected: *Teucrium dunense*, *Helichrysum stoechas*, and *Crucianella maritima*. These species exhibit divergent ecological strategies across the substrate stability gradient and dominate distinct sectors of EU habitats 2210 (*Crucianellion maritimae* fixed beach dunes) and 2260 (Cisto-Lavanduletalia dune sclerophyllous scrubs). *C. maritima* is a specialist of less stabilised soils, *T. dunense* typically inhabits fist stabilized areas, and *H. stoechas* co-occurs with the former but predominates in disturbed or unstable patches.

From a phytosociological perspective, *T. dunense* and *H. stoechas* are associated with the Halimionenion halimifolii suballiance—specifically within the *Teucrio dunensis-Helianthemetum capitis-felicis* association and its *H. stoechas* facies (EU Habitat 2260). *C. maritima* is diagnostic of the Crucianellion maritimae (*Loto cretici-Crucianelletum maritimae*) (EU Habitat 2210).

Leaf litter from *T. dunense* and *H. stoechas* was collected from at least 10 individuals per species during the last week of August, prior to the first late-summer rains, at the Sa Ràpita dune system (Mallorca, 39°

21' 44" N, 2° 57' 35" E). *C. maritima* litter was sampled at Platja de Llevant (Formentera, 38° 45' 12" N, 1° 26' 4" E).

Fresh material was air-dried at room temperature and ground into a homogeneous powder prior to extraction. To assess temporal changes in litter effects, subsamples were collected at 0, 3, 7, 10, and 15 months. Germination bioassays with aged litter were performed at selected stages according to their ecological relevance within the annual cycle. The 300-day litter was used to evaluate whether phytotoxicity persisted before completion of a full reproductive cycle, whereas the 15-month litter (450 days) was used to test whether delayed germination responses remained after that cycle had elapsed.

Seeds of *C. maritima* and *H. stoechas* were collected from the same populations. *T. dunense* seeds were sourced from the previous year's collection, as peak germination and optimal response times for this species are achieved with seeds aged 300–450 days (Gil, 1994).

2.2. Aqueous extract preparation and phytotoxicity bioassays

Aqueous extracts were prepared by soaking 75 g of dry leaf litter in 1 L of distilled water, followed by 36 h of orbital shaking (Cavieres et al., 2007). To ensure reproducibility, suspended matter was quantified via rotary evaporation, and the dry residue was determined by evaporating 5 mL aliquots at 75°C until reaching constant weight.

Bioassays were conducted using three concentrations: 100%, 35%, and 10% of the base extract, corresponding to 75, 26.25, and 7.5 g L^{-1} of dry litter, respectively. Germination tests were performed at 20°C, the optimal temperature for all three species (Gil, 1994), using square Petri dishes (9 × 9 cm) lined with a sterile gauze base saturated with 5 mL of either the extract solution or distilled water (control). For each treatment, 20 seeds were sown per dish with five replicates (n = 100; ISTA, 2007). The dishes were incubated for 30 days in climatic chambers (p-Selecta Hotcold-GL/UM). To assess the reversibility of inhibition, non-germinated seeds were subsequently transferred to dishes with distilled water under the same environmental conditions. Recovery percentage (%R) was calculated as the proportion of previously ungerminated seeds that germinated after transfer to distilled water: %R = (seeds germinated after transfer / seeds ungerminated at the end of the extract treatment) × 100.

To evaluate the effect of BVOCs emitted by living plants, *T. dunense* individuals were placed inside the germination chamber alongside five uncovered Petri dishes, each containing 20 seeds of the recipient species, ensuring no physical contact. The air was refreshed every 24 h to prevent the excessive accumulation of reactive compounds. Hermetically sealed dishes (Parafilm) served as the control. Incubation was maintained at 20°C with a 12/12 h light/dark photoperiod.

2.3. Quantification and chemical analysis of the volatile fraction

2.3.1. Extraction and quantification

The methodology followed Hassiotis (2010) and Hassiotis & Lazari (2010). Each determination was performed in duplicate using 2 g of dry leaf litter. Samples were placed in glass pipettes plugged with cotton and washed three times with 5 mL of diethyl ether (15 mL total volume). The resulting solution was evaporated in a fume hood at room temperature for 48 h. Yield was expressed relative to dry weight, following moisture content determination by oven-drying at 75°C for 48 h.

2.3.2. Distillation, isolation, and characterization (GC-MS)

BVOCs were isolated via simultaneous distillation-extraction (SDE) using a Likens-Nickerson apparatus (Likens & Nickerson, 1964), according to the modifications by Llorens et al. (2014) and Schultz et al. (1977). Extracts were analyzed using a Clarus 500 GC-MS (PerkinElmer) equipped with a ZB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The temperature program started at 65°C (2 min), followed by a ramp of 4°C min^{-1} up to 220°C. Compound identification was based on retention indices (RI) and comparison with the NIST 08.2.4 library.

2.3.3. Analysis of BVOCs in germination chamber.

The atmosphere within the germination chambers was characterized using Headspace Solid-Phase Microextraction (HS-SPME), adapting the protocol described by Tomàs et al. (2022). To sample the volatiles to which the target seeds were exposed, the air from the chambers was equilibrated in 10 mL glass vials at 25°C for 20 min. A Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) fiber (Supelco Inc., Bellefonte, USA) was then exposed to the headspace for 30 min at 20°C. Subsequent analyses were performed using an Agilent 6980 GC - MSD 5975 inert XL equipped with a Supelcowax 10 capillary column (60 m × 0.25 mm × 0.25 µm). The injector and detector temperatures were set at 250°C. The oven temperature program increased from 50°C to 250°C at a rate of 5°C min^{-1}. Compound identification was carried out by comparing Kovats retention indices (relative to C7 -C30 alkanes) with the NIST MS Search 2.0 library.

2.4. Statistical analysis

For each recipient species and treatment set, final germination percentage and T50 were analyzed using one-way ANOVA. Percentage data were arcsine-square root transformed (y = arcsinx) to meet the assumptions of normality and homogeneity of variance. Mean separations were performed using Tukey's HSD test ($p < 0.05$).

3. RESULTS

3.1. Interaction matrix and inhibitory hierarchy under aqueous extract exposure

Germination responses differed among donor species and extract concentrations, revealing clear interspecific differences in inhibitory activity (Table 1). In general, inhibition increased with concentration, with the strongest effects usually observed at 75 g L^{-1}. Significant differences from the water control are indicated by asterisks in Table 1 ($p < 0.05$).

Table 1
Final germination percentage of recipient species exposed to T = 0 aqueous extracts from different donor species. Rows represent recipient species (Crucianella maritima, CM; Helichrysum stoechas, HS; Teucrium dunense, TD), and columns represent donor species and extract concentration. Extract concentrations: A = 7.5 g L^{-1}, B = 26.25 g L^{-1}, and C = 75 g L^{-1}. Asterisks (*) indicate significant differences from the water control ($p < 0.05$).

		Extract concentration								
		CM			**HS**			**TD**		
Recipient Species		**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
CM	**Germination (%)**	88.0*	86.0*	78.0*	89.0*	85.0*	68.0*	38.3	31.6*	20.0*
HS		62.5*	54.2*	25.0*	56.2*	44.8*	21.9*	52.1	39.6	24.0*
TD		96.0	88.0*	68.0*	80.0*	71.7*	35.0*	71.6	75.0	38.3*

Among the donor species, *T. dunense* consistently exerted the strongest inhibitory effects, particularly at the highest concentration, reducing germination to 20.0% in *C. maritima* and 24.0% in *H. stoechas*. At the intermediate concentration, inhibition remained marked for *C. maritima* (31.6%), whereas the response of *H. stoechas* was weaker and not always statistically significant. These results identify *T. dunense* as the species with the greatest inhibitory activity in the aqueous extract assays.

H. stoechas showed intermediate inhibitory activity. Its extract notably affected *C. maritima*, whose germination declined to 68.0% at the highest dose, while the effects on *T. dunense* were generally weaker at low and intermediate concentrations, although a substantial reduction was still observed at the maximum dose (35.0%). In contrast, *C. maritima* showed the weakest overall inhibitory effect, with comparatively limited reductions in most donor–recipient combinations.

Recipient species also differed in their tolerance to heterospecific extracts. *T. dunense* was the relatively most tolerant receptor, generally maintaining higher germination values than the other species when exposed to extracts from *C. maritima* and *H. stoechas*. By contrast, as noted above, *C. maritima* and *H. stoechas* were more sensitive to *T. dunense* extract. Furthermore, autotoxic effects were especially evident in *H. stoechas*, whose germination decreased to 21.9% at the maximum concentration of its own extract. A similar but less pronounced reduction (38.3%) was observed in *T. dunense* when exposed to its own extract..

Overall, the interaction matrix revealed a consistent inhibitory hierarchy, with *T. dunense* as the most effective donor species, *H. stoechas* showing intermediate activity, and *C. maritima* exhibiting the weakest inhibitory effect. Because *T. dunense* showed the strongest and most consistent inhibition

across receptor species, subsequent analyses of litter persistence and volatile emissions focused on this species.

3.2. Aqueous extract yield and temporal depletion of the volatile fraction

The amount of solid material recovered in the aqueous extracts varied markedly among species, with *T. dunense* showing the highest extraction yield (2.4%, w/v), exceeding those obtained for *H. stoechas* (0.9%) and *C. maritima* (0.7%). This result indicates a greater transfer of litter-derived material from T. dunense into the aqueous phase, although the ecological relevance of this pattern depends on the chemical identity and bioactivity of the extracted compounds.

3.3. Evolution of phytotoxicity: Germination kinetics, litter persistence and T50 delay

3.3.1. Alteration of germination kinetics and persistence of phytotoxicity in 300-day litter

Aged litter bioassays were first evaluated at 300 days, a stage chosen to represent prolonged litter persistence before completion of a full annual reproductive cycle. The application of *T. dunense* extracts altered the germination kinetics of all three recipient species (Figs. 2 and 3). In fresh litter extracts (T = 0; Fig. 2), increasing concentration produced a progressive rightward shift of the germination curves and, in most cases, a reduction in their slope and final germination. This pattern was especially pronounced in *H. stoechas*, which showed the strongest inhibition at intermediate and high concentrations, and in *C. maritima*, where the highest dose (75 g L^{-1}) caused a marked delay and reduction in final germination. *T. dunense* also showed delayed germination under its own extracts, with the strongest effect again observed at 75 g L^{-1}.

Analysis of aged litter extracts (T = 300; Fig. 3) showed that the inhibitory activity of *T. dunense* was retained after decomposition. In *C. maritima* and *H. stoechas*, the effects of aged litter were generally weaker than those observed for fresh extracts, particularly at low and intermediate concentrations, although a clear inhibitory response was still evident at 75 g L^{-1}. In *T. dunense*, the aged extracts also maintained a concentration-dependent effect, with the highest dose producing the strongest delay and lowest final germination, consistent with persistent autotoxicity.

Overall, these results indicate that the phytotoxic effects of *T. dunense* litter persist after 300 days of decomposition, although their intensity is reduced in some donor–recipient combinations relative to fresh litter.

3.3.2. T50 delay

To determine whether delayed germination responses persisted beyond a full annual reproductive cycle, T50 values were compared between fresh litter extracts (T = 0) and litter stored for 15 months (T = 450 days) (Fig. 4). In fresh extracts, T50 increased with concentration in *C. maritima* (from 3 to 6.5 days), *H. stoechas* (from 4 to 9 days), and *T. dunense* (from 9 to 16 days). A similar pattern was observed for aged litter extracts, with T50 values rising from 5.5 to 11 days in *C. maritima*, from 7 to 18 days in *H. stoechas*, and from 17 to 21 days in *T. dunense*. These results indicate that *T. dunense* litter not only reduced final germination, but also delayed germination timing, and that this effect persisted after prolonged litter aging.

3.4. Chemical characterization of leaf litter

The chemical evidence supports the biological dominance observed in the previous sections. The litter of *T. dunense* displayed a much more complex and dynamic chemical volatile profile, representing a persistent chemical legacy. As shown in Supplementary Table 1 (S1), the high initial concentration of phytotoxic monoterpenes during the first stages of decomposition (September and December) provides a clear chemical explanation for its superior inhibitory capacity. Furthermore, the strong correlation between these litter compounds and the emissions identified in the germination chamber (see 3.6) reinforces the role of BVOCs in the long-term interference patterns of the dune ecosystem.

In contrast, the volatile profile of *H. stoechas* litter was relatively stable but limited in diversity, characterized mainly by α-pinene β-caryophyllene and Alloaromadendrene (Supplementary Table 2 (S2**)**.

3.5. Reversibility tests

Reversibility tests conducted with both fresh (T=0; Fig. 5) and aged (T=300; Fig. 6) litter extracts showed generally low recovery across all species and concentrations after transferring non-germinated seeds to distilled water. *C. maritima* exhibited the highest recovery in both cases, particularly at the lowest concentration (7.5 g L−1), where it reached 25%with fresh extracts and 33.3% with aged extracts; however, recovery declined sharply or vanished at higher doses. In contrast, *H. stoechas* and *T. dunense* showed minimal to null recovery (0–5.5%) regardless of extract age or concentration, with *T. dunense* only showing a slight, detectable recovery (1.5–3.8%) when exposed to aged litter. These results indicate that the inhibitory effects of *T. dunense* extracts are largely persistent and only weakly reversible, supporting the view that its phytotoxicity induces long-term suppression of germination rather than a transient delay.

3.6. Phytotoxicity of *Teucrium dunense* BVOCs

Exposure to BVOCs emitted by living *T. dunense* plants significantly reduced the final germination of *H. stoechas* and *C. maritima* (Fig. 7). Final germination declined from approximately 97% to 58% in *H. stoechas* and from 98% to 68% in *C. maritima*, whereas *T. dunense* showed no detectable reduction in final germination under BVOC exposure (60% in the control vs. 61% under BVOCs).

BVOC exposure also delayed germination kinetics in all three species. T50 increased from 3 to 9 days in *C. maritima*, from 10 to 12 days in *H. stoechas*, and from 6 to 12 days in *T. dunense*. Thus, even when final germination was unaffected, as in *T. dunense*, volatile exposure still altered the timing of germination.

3.7. Volatile profile of the germination chamber atmosphere

The analysis of the air within the germination chambers revealed a specific chemical "fingerprint" that explains the inhibitory effects observed during the exposure trials. This atmosphere, generated by the donor plants (*T. dunense*), was characterized by a high prevalence of low-molecular-weight monoterpenes.

As shown in Table S1, the most abundant compound in the chamber was α-pinene (13.1 relative area), followed by sabinene (3.5) and β-myrcene (2.1). The presence of these specific BVOCs in the gaseous phase confirms that the target species were subjected to a continuous flux of phytotoxic molecules throughout the germination process. This BVOC-rich atmosphere in the chamber headspace provides support for a mechanistic role of volatile compounds in the germination inhibition observed in the previous sections.

4. DISCUSSION

Results from this study demonstrate that chemical ecology plays a decisive role in structuring plant communities within Mediterranean backdunes, complementing and occasionally modulating the influence of abiotic gradients traditionally regarded as primary determinants. The strong inhibition exerted by *T. dunense* extracts on *H. stoechas* and *C. maritima*—with reductions exceeding 60% at higher concentrations—reveals a strong inhibitory capacity. This is consistent with the high concentrations of bioactive monoterpenes and sesquiterpenes described for this genus (Hassiotis, 2010; Kurtoğlu & Tin, 2017). The tolerance of *T. dunense* to its own compounds suggests detoxification or compartmentalization mechanisms, which are characteristic traits of dominant allelopathic species (Inderjit et al., 2011).

These findings help explain its ability to establish dense stands in more stable areas of the dune gradient (EU habitat 2260). Once established, *T. dunense* appears to function as a biotic filter; its potent inhibitory capacity and the long-term persistence of its chemical legacy create a "chemical barrier" that limits the settlement of other shrubs, effectively maintaining its dominance and stabilizing the vegetation mosaic in the most fixed areas of the backdune.

The behavior of companion species reinforces this functional interpretation. *H. stoechas*, exhibiting moderate inhibitory effects alongside marked autotoxicity—reducing its own germination in concentrated extracts, a phenomenon widely documented in species rich in volatile compounds (Singh et al., 1999)—points to a dual role within the community. On one hand, it may limit the germination of

sensitive competitors; on the other, its own vulnerability could restrict its expansion in the absence of favorable environmental conditions. This self-limitation suggests that *H. stoechas* behaves as an opportunistic pioneer species within this system, taking advantage of areas or periods with greater disturbance. In these microhabitats, substrate mobility prevents the excessive buildup of its own allelopathic litter, allowing it to thrive where other species cannot settle, although it loses its competitive edge as the site stabilizes.

In contrast to *T. dunense* and *H. stoechas*, *C. maritima* displayed the lowest allelopathic activity. This pattern is consistent with its ecological strategy associated with shifting sand environments, where chemical competition is less relevant than tolerance to physical stress—a trait widely described in Mediterranean species adapted to limiting conditions (Gratani et al., 2004). The success of *C. maritima* in these early successional stages (semi-mobile dunes) is likely due to its high abiotic tolerance rather than competitive exclusion. As the dune stabilizes and more competitive or allelopathic species like *T. dunense* arrive, *C. maritima* is displaced toward the front, where lower shrub density reduces its exposure to a chemical interference it is not equipped to fight.

Nevertheless, the temporal dimension of chemical interference emerges as a key element in backdune dynamics. *T. dunense* litter maintained notable phytotoxic activity after 300 days, even as its chemical composition underwent substantial changes. The progressive disappearance of hydrogenated monoterpenes and the increase in oxygenated sesquiterpenes align with patterns described in the degradation of terpene-rich litter (Zhang et al., 2008; Mishra & Kumar, 2016). The persistence of biological activity suggests that oxidation products or microbial transformation metabolites maintain—or even enhance—phytotoxicity, as observed in other Mediterranean systems (Cheng & Cheng, 2016; Kainulainen & Holopainen, 2002). This legacy effect, coupled with the germination delay induced by secondary metabolites under stress conditions (Khan et al., 2025), implies that the influence of *T. dunense* extends beyond the life of its tissues, modulating plant succession and contributing to the stability of vegetation mosaics in the backdune.

Assays with BVOCs emitted by *T. dunense* add a further spatial dimension to this framework. Emissions in the germination chamber, dominated by monoterpenes such as α-pinene (13.1 relative area), sabinene, and β-myrcene reduced the germination of *H. stoechas* and *C. maritima*, while the emitting species showed no negative effects. These compounds, involved in plant-plant communication, chemical defense, and soil microbiome modulation (Mofikoya et al., 2019; Rosenkranz et al., 2021; Jin et al., 2023; Kessler et al., 2023), extend the zone of influence of *T. dunense* beyond direct soil contact, reinforcing its role as a habitat-structuring species.

Taken together, the results reveal a system in which chemical processes operate across multiple scales and pathways—extracts, volatile emissions, and litter—generating a functional hierarchy among species that contributes to the zonation patterns characteristic of Habitats 2210 and 2260. The chemical interference of *T. dunense*, the autotoxicity of *H. stoechas*, and the vulnerability of *C. maritima* configure a dynamic mosaic where coexistence and spatial distribution cannot be explained solely by abiotic

factors. Chemical ecology thus emerges as a key structuring component, with immediate and legacy effects that influence regeneration, succession, and system stability.

These findings have direct implications for the conservation and management of Natura 2000 habitats. The loss of spatial heterogeneity or alterations in disturbance regimes may modify the intensity and distribution of allelopathic processes, affecting system coexistence and resilience (Prisco et al., 2016; Malavasi et al., 2016). Integrating chemical ecology into restoration strategies could improve the effectiveness of interventions, especially within the context of land-use and climate change (Pinna et al., 2022).

5. CONCLUSION

This study demonstrates that chemical ecology represents an important structuring mechanism in Mediterranean dunes, operating through multiple pathways—extracts, volatile emissions, and litter—that directly influence seed germination, establishment, and species coexistence. The potent inhibitory capacity of *T. dunense*, combined with its self-tolerance, explains its dominance in the more stable areas of Habitat 2260. Conversely, the autotoxicity of *H. stoechas* and the high sensitivity of *C. maritima* contribute to their restricted distribution along the dune gradient. The persistence of phytotoxic activity in the litter, even after substantial chemical transformations, reveals a legacy effect that extends the influence of *T. dunense* beyond the lifespan of its tissues.

Taken together, these results indicate that chemical processes not only modulate plant–plant interactions but also contribute decisively to zonation patterns and the stability of Habitats 2210 and 2260. Integrating chemical ecology into conservation and restoration strategies will enhance the management of these fragile systems within a context of increasing anthropogenic pressure and climate change.

Declarations

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Google Gemini to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the final manuscript.

Author Contribution

Author Contributions: B.P. and L.Ll. designed the study, performed the experiments, and analyzed the data. L.G. contributed to the methodology and assisted with data collection and experimental

procedures. L.Ll. drafted the manuscript. All authors participated in the writing, and reviewed and approved the final manuscript.

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Figures

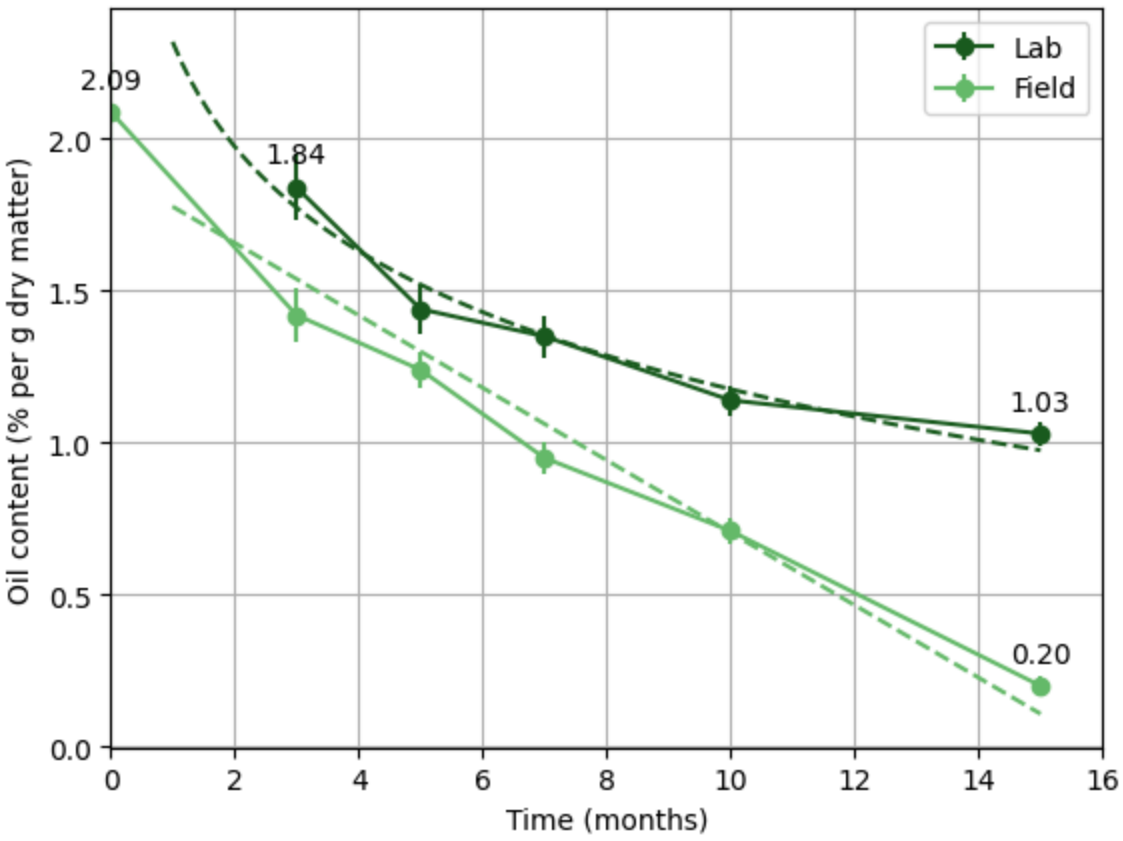

Figure 1

Dynamics of biogenic volatile organic compound (BVOC) depletion in *Teucrium dunense* litter. Exponential retention is observed in the laboratory (dark green, 1.03% remaining), contrasting with pronounced linear loss in the field (light green, 0.20% remaining). Symbols represent means ± Standard Error (SE); dashed lines represent fitted regression models. The time axis covers 15 months, equivalent to the T = 0 to T = 450 days period mentioned in the text.

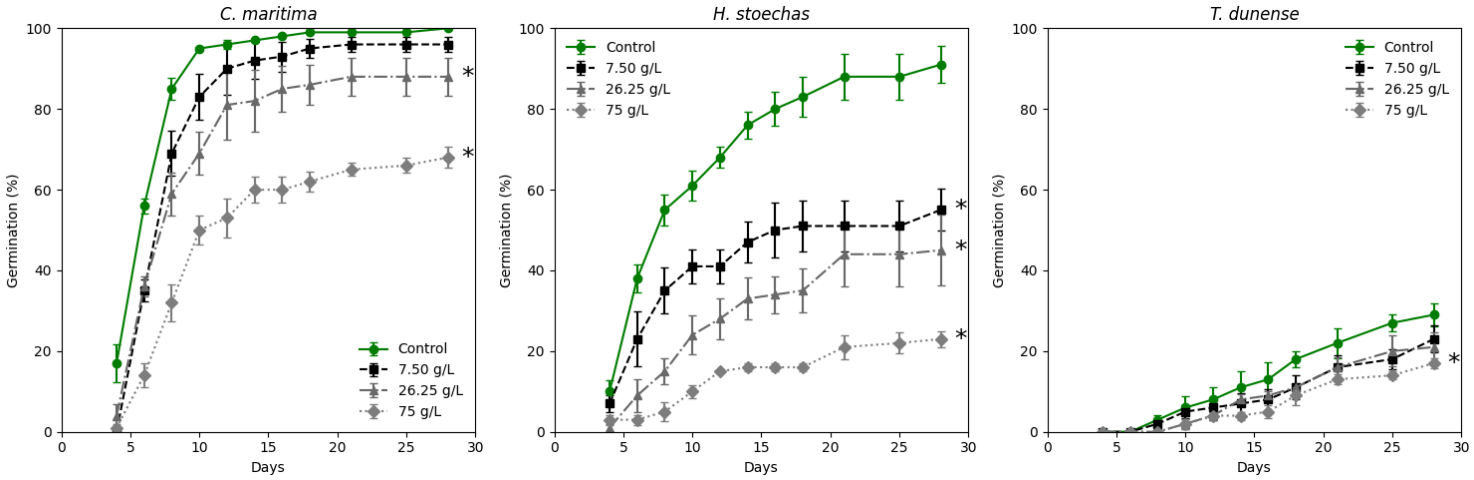

Figure 2

Effects of *Teucrium dunense*extracts on germination of *Crucianella maritima*, *Helichrysum stoechas*and *T. dunense*at 0 days. Asterisks (*) denote significant differences ($p < 0.05$) compared to the control.

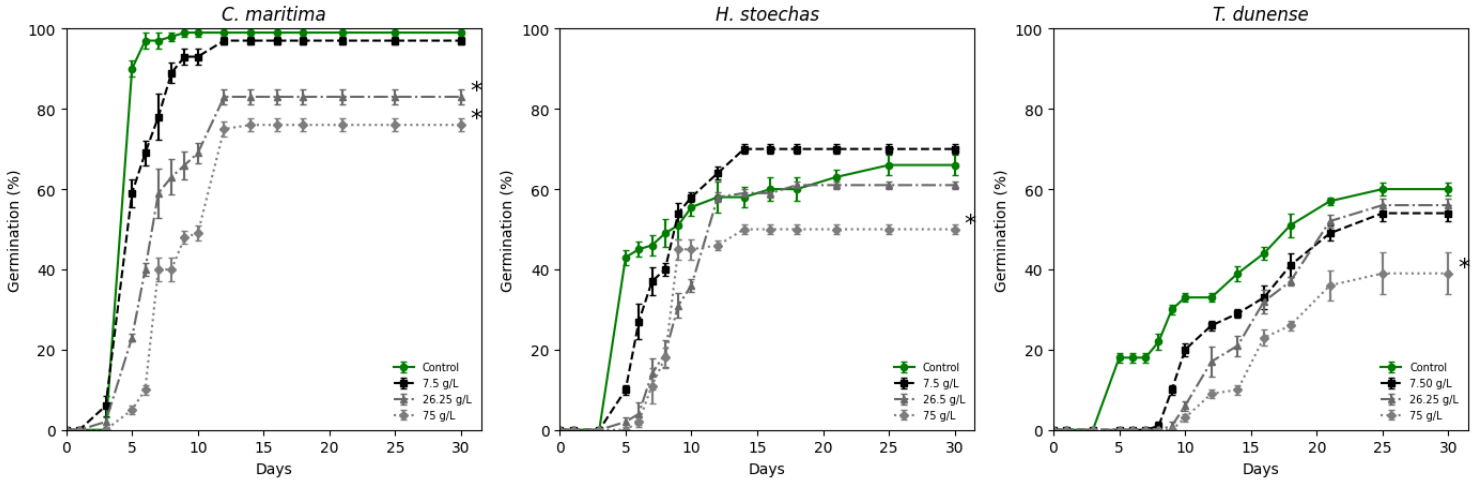

Figure 3

Effects of *Teucrium dunense*extracts on germination of *Crucianella maritima*, *Helichrysum stoechas*and *T. dunense*at 300 days. Asterisks (*) denote significant differences ($p < 0.05$) compared to the control.

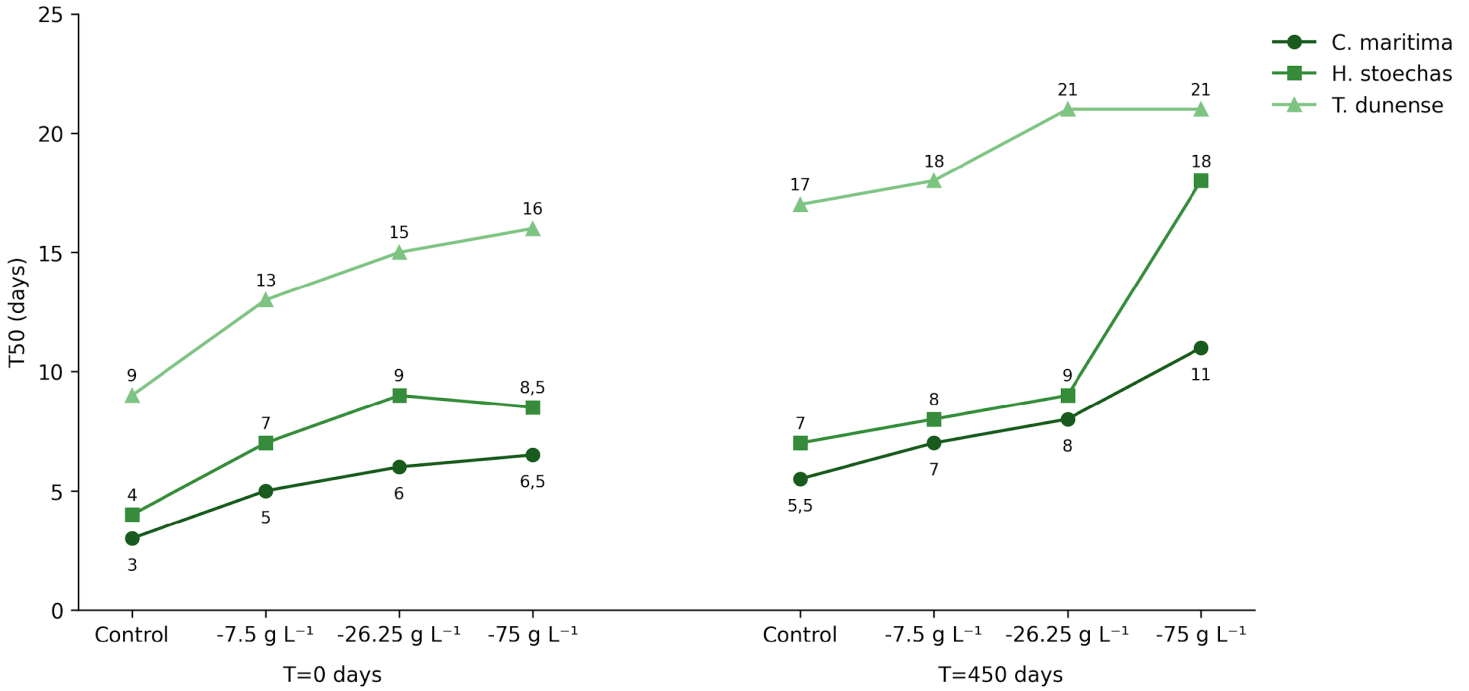

Figure 4

Comparison of germination speed (T50) using aqueous extracts obtained from fresh litter (T = 0 days) and litter stored for fifteen months (T = 450 days). Values indicate the time required to reach 50% of the maximum germination for *Crucianella maritima*, *Helichrysum stoechas*, and *Teucrium dunense*.

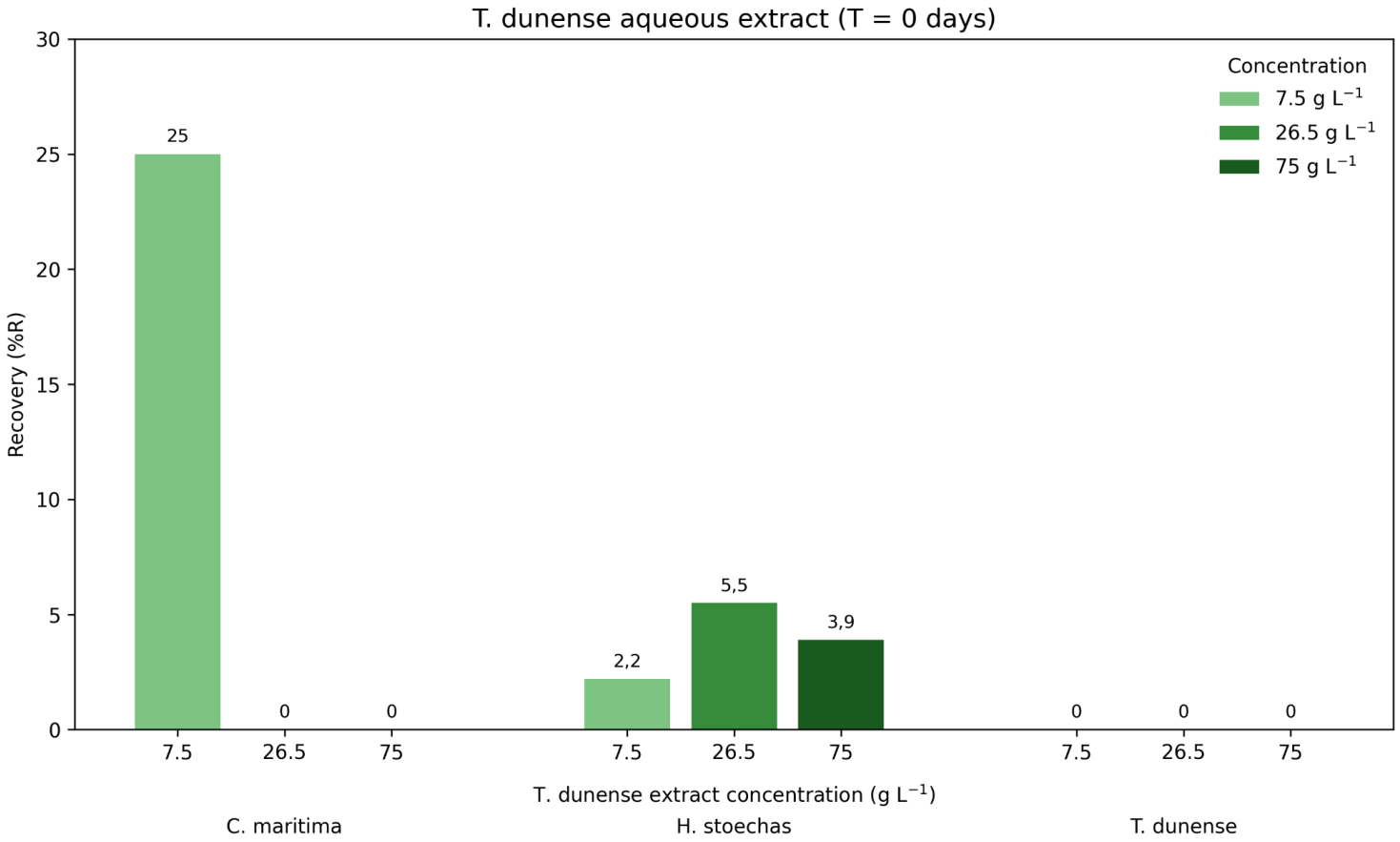

Figure 5

Recovery percentage (%R) of previously ungerminated seeds after transfer to distilled water following exposure to aqueous extracts of *T. dunense* litter at T = 0 days. %R was calculated relative to the number of seeds that remained ungerminated at the end of the extract treatment.

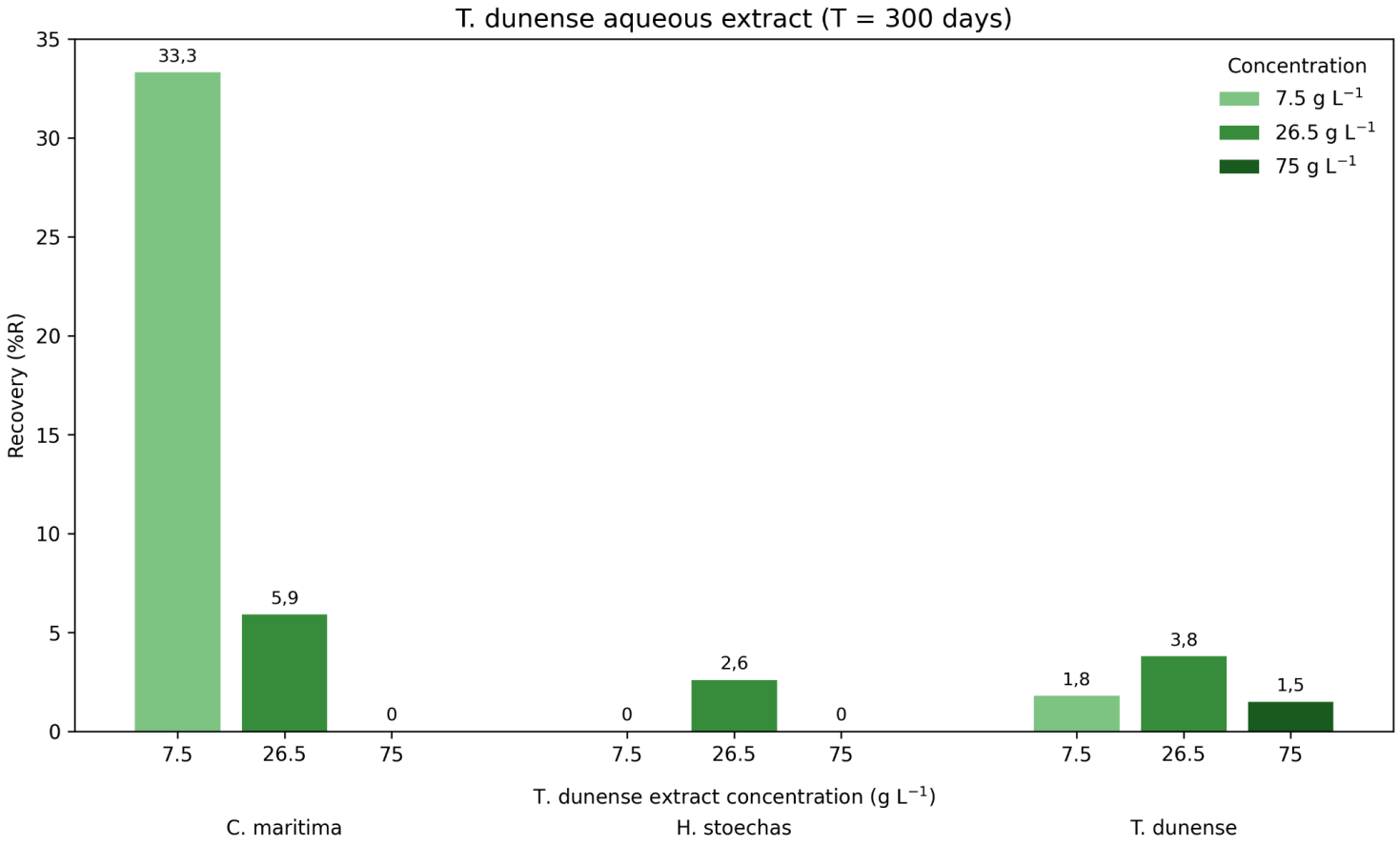

Figure 6

Recovery percentage (%R) of previously ungerminated seeds after transfer to distilled water following exposure to aqueous extracts of *T. dunense* litter at T = 300 days. %R was calculated relative to the number of seeds that remained ungerminated at the end of the extract treatment.

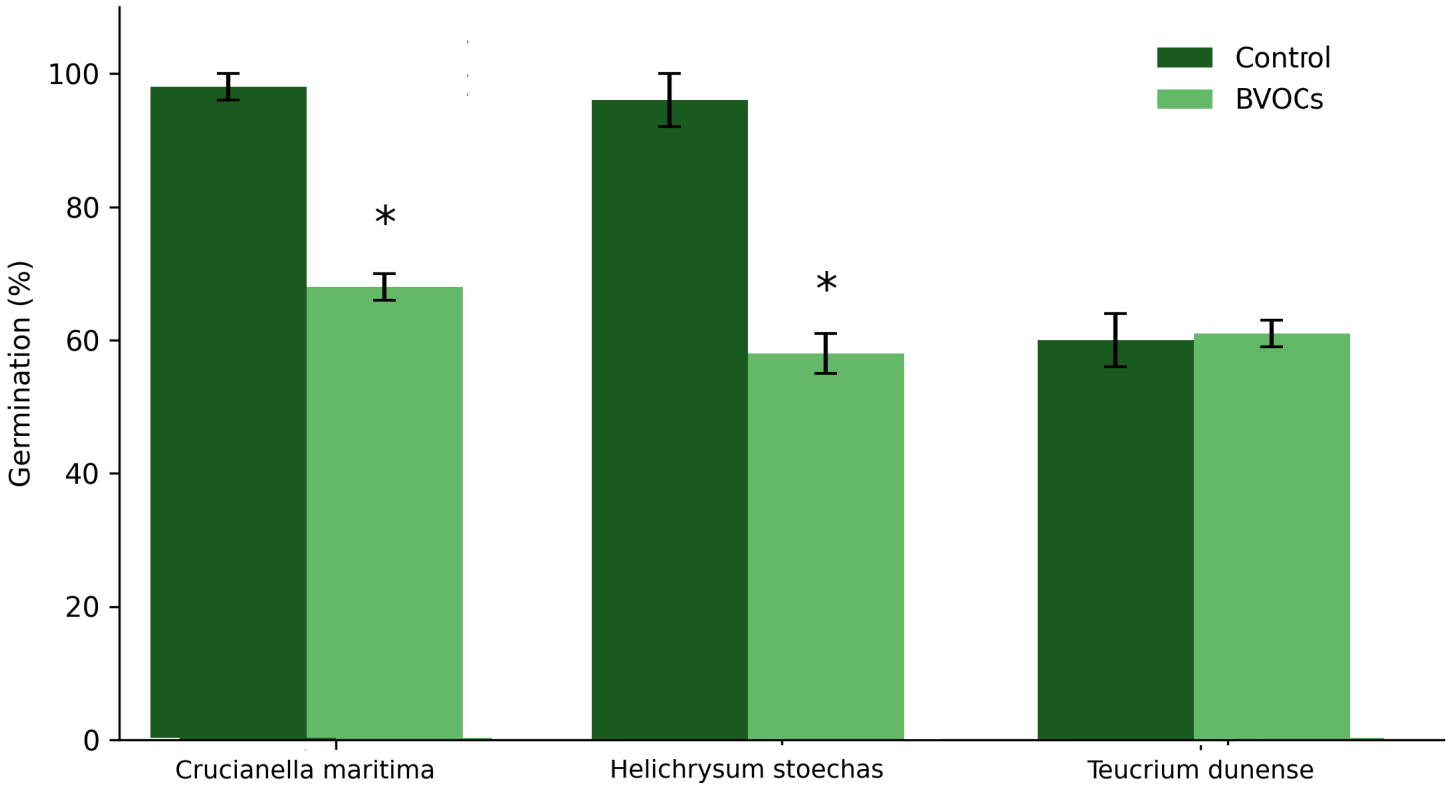

Figure 7

Final germination percentage of *Crucianella maritima, Helichrysum stoechas*, and *Teucrium dunense*after exposure to BVOCs emitted by *T. dunense*.

Supplementary Files

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